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Enhanced sulfide removal and bioelectricity generation in microbial fuel cells with anodes modified by vertically oriented nanosheets

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Enhanced sulfide removal and bioelectricity generation in microbial fuel cells with anodes modified by vertically oriented nanosheets

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Abstract

Anode materials and structures are of critical importance for microbial fuel cells (MFCs) recovering energy from toxic substrates. Carbon-fiber-felt anodes modified by layers of vertically oriented TiO₂ and Fe₂O₃ nanosheets respectively were applied in present study. Enhanced sulfide removal efficiencies (both over 90%) were obtained after 48 h operation, with maximum power densities improved by 1.53 and 1.36 folds compared with MFCs with raw carbon-fiber-felt anode, respectively. The

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modified anodes provided more active sites for microbial adhesion with increasing biomass densities. High-throughput 16S rRNA gene sequencing analysis also indicated the increase of microbial diversities. Bacteroidetes responsible for bioelectricity generation with *Thiobacillus* and *Spirochaeta* dominating sulfide removal were found in the MFCs with the modified anodes, with less anaerobic fermentative bacteria as Firmicutes appeared. This indicates that the proposed materials are competitive for applications of MFCs generating bioelectricity from toxic sulfide.

Keywords: Sulfide; Nano-sheets; Microbial fuel cells; Microbial community; Carbon-fiber-felt

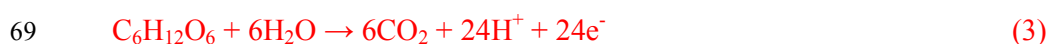
1. Introduction

Sulfide is a hazardous, corrosive and odorous substance that often occurs in industrial wastewaters, especially in effluent from viscose rayon industries, petrochemical plants and tanneries [1,2]. Sulfide is toxic to human health, with studies showing that sulfide is particularly harmful to cytochrome c oxidase and causes cell hypoxia [3]. Thus, sulfide-contaminated wastewater must be treated thoroughly before discharge into the environment. Although common physical-chemical methods, such as adsorption and chemical oxidation, can remove sulfide, they are costly to implement and require high energy inputs [4]. In contrast, biological processes provide an environmental-friendly alternative for sulfide removal from both liquids and gases under ambient environmental conditions [5-7].

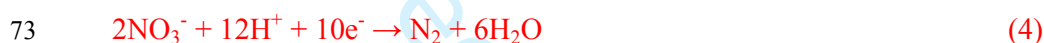
Microbial fuel cells (MFCs) are nowadays recognized to be renewable, clean energy sources that can generate bioelectricity during wastewater treatment [8,9]. Numerous pollutants have been successfully handled with energy recovery based on MFC technology, including organics and metals [10-12]. Additionally, anode materials and structures are particularly important regarding the performance of MFCs in removal of toxic substances: properly designed anodes can help electricigens to collect electrons from anolyte and thus sustain bioelectricity generation while tolerating harmful substrates [13]. Carbon-based anodes are most frequently used [14] and further gains in performance arise from coating them with conducting polymers or metal oxides [15,16]. Nowadays, hydrothermally synthesized layers of vertically oriented metal oxides nanosheets such as TiO_2 and Fe_2O_3 with activated carbon (AC) powder in situ on the surface of carbon paper exhibit excellent behavior as MFCs' anodes as they have high biocompatibility, support mass transport, have large surface area for adhesion of bacteria, and provide direct pathways for electrons movement to the electrode [17,18]. Furthermore, carbon fiber felt is more promising as anodic material for scale-up of MFCs because it offers a more suitable support for bacteria attachment and growth, higher mechanical strength, more active sites for the chemical reactions [19-21], while its performances after being modified by layers of vertically oriented metal oxides nanosheets are rarely reported. **Studies have been carried out on sulfide removals using MFCs [4,22,23]. In anode chambers, sulfide acts as electron donor and it is biochemically oxidized based on Equ. (1) and (2), with anodic electrodes as electron acceptors:**



Commonly, co-substrate such as glucose is supplied as co-existing electron donor [4], which is oxidized as Equ. (3),



Moreover, other contaminants with higher redox potentials such as nitrate may be presented with sulfide [22]. It can also act as competitive electron acceptor, which is reduced as Equ. (4),



Then electrons flow to cathode via external circuits. In cathode chambers, oxygen and potassium ferricyanide are most frequently used as electron acceptors and they are reduced as Equ. (5) and (6) [23,24],



While most of these studies focus on co-existing pollutants removals [4,22,24-26], operating factors investigations [27] and commercial electrodes comparisons [28]. Little attention has been paid to date on modified carbon-based anode especially for carbon fiber felt to enhance bioelectricity generation and sulfide removal in the context of accumulation of microbes in anode chambers with toxic matrix.

The present research explores carbon-fiber-felt anodes modified with two kinds of metal oxides (TiO_2 and Fe_2O_3) nanosheets respectively that are vertically oriented on the surface. Enhanced performances in aspects of power outputs and sulfide removals

are investigated for MFCs equipped with these anodes, compared with unmodified one. The amounts and species of accumulated microbes are also analyzed. This work provides a potential alternative for treating sulfide-contaminated wastewater by MFC technology with proposed anodes.

2. Materials and methods

2.1 Preparation of anodes and fabrication of MFCs

TiO₂ and Fe₂O₃ sols were synthesized in the laboratory following a previous report [17,18]. Clean carbon-fiber-felt materials (3 mm thickness, 4 cm length and width, provided by Beijing Evergrow Resources CO., LTD, China) were immersed in the TiO₂ and Fe₂O₃ sols for 10 min and dried at 80 °C. After that, the materials were calcined for 30 min in a tubular furnace at 350 °C so that a TiO₂ and Fe₂O₃ seed layers formed on the surfaces of the carbon-fiber felts. A Teflon-lined stainless steel autoclave (50 mL in volume) filled with 40 mL of aqueous solution of 10 M NaOH and 0.2 g of AC powder was placed in an oven at 180 °C for 24 h. After the carbon-fiber-felts had cooled down to room temperature, the modified carbon-fiber felts were rinsed with ultrapure water to remove AC, followed by soaking with 0.1 M hydrochloric acid for 1 h, then washed to neutral with deionized water and dried at 80 °C. Sequentially, the samples were calcined at 550 °C for 1 h in a N₂ atmosphere [17,18].

Three H-type MFCs with cylindrical chambers (250 mL for each) were fabricated as previously described [29]. The two chambers for each MFC were divided by a

proton exchange membrane (Nafion117#, Dupont, USA) with a surface area about 4 cm². The MFCs were equipped with TiO₂ nanosheets modified carbon-fiber-felt anode (MFC-TiO₂) and Fe₂O₃ nanosheets modified carbon-fiber-felt anode (MFC-Fe₂O₃), respectively, as prepared above, with surface area of 16 cm². Raw carbon-fiber-felt anode without modification was also employed as control (MFC-CF). All cathodes were made of ordinary carbon-fiber-felt with the same size as anodes. The anode and cathode were connected across a 100 Ω external resistor. Each anode chamber was inoculated with 25 mL anaerobic granular sludge from an up-flow anaerobic sludge blanket (UASB) reactor treating high strength sulfate wastewater. The anolyte included the following (per L): 0.75 g of C₆H₁₂O₆; 5.62 g of NaH₂PO₄·2H₂O; 6.15 g of Na₂HPO₄·12H₂O; 0.31 g of NH₄Cl; 0.13 g of KCl; 1.25 mL of vitamin solution; and 12.5 mL of trace mineral element solution. Sulfide was added to the anode solution in the form of Na₂S·9H₂O with concentration of 60 mg L⁻¹ to facilitate the comparison [30]. The catholyte included the following (Per L): 9 g of KH₂PO₄, 8 g of K₂HPO₄·3H₂O and 0.05 g K₃[Fe(CN)₆].

2.2. Experimental procedures

Morphology and composition of the modified anodes were analyzed first. Then successful start-up of all MFCs was realized by refreshing anolyte every 2 days. After that, the power outputs and sulfide removals in a typical cycle (48 h) were evaluated, noting that most of the sulfide was removed within that time. Electrochemical measurements were also undertaken for the three types of MFC. After 3 months

operation with refreshment of anolyte every 2 days, ultrasonic was employed to collect the bacteria attached to the surfaces of the anodes, and the samples then used for high-throughput 16S rRNA gene sequencing on MiSeq (Illumina, the USA). All experiments were carried out at room temperature (22 ± 2 °C) for practical application consideration and easy comparison with existing studies [22]. For each condition, trials were carried out in triplicate and average results from the three MFCs were reported.

2.3. Analytical methods and data representation

Measurement of chemical oxygen demand (COD) was undertaken based on digestion with potassium dichromate in concentrated sulfuric acid for 2 h at 150 °C. Sulfide was determined according to the methylene blue method ($n = 665$ nm) [31]. The indication of “sulfide” described all species (H_2S , HS^- , and S^{2-}). Total organic carbon (TOC) was monitored by Multi N/C 3000 TOC analyzer (Analytik Jena AG, Germany). Sulfate was measured by ion chromatography (ICS-1600, Dionex, the USA). pH was measured using a pH-201 meter (Hanna, Italy). The biomass on the anode surface was determined using the phospholipid analysis as previously described and the biomass density was expressed as the mass of phosphorus normalized by anode volume (48 cm^3) [32]. The morphology and composition of modified anodes were analyzed by a JEOL JAX-840 scanning electron microscope (SEM) operating at 20 kV with an energy dispersive X-ray (EDX).

Voltage measurements were taken using a voltmeter throughout the test. The

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4 150 polarization curve and power outputs were obtained by varying the external resistance
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6 151 over the range from 10 to 5000 Ω . For each point on the polarization curve, readings
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8 152 were taken when pseudo-steady-state conditions was established, which might take
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10
11 153 several minutes or more [4,33,34]. MFCs operated at least twice under each resistance
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13 154 to ensure the repeatability of power outputs. Power density was normalized to the
14
15 155 single-side projected surface area of the anode. Cyclic voltammetry (CV)
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18 156 measurement was carried out at a scan rate of 2 mV s⁻¹ in the range of -1 V to +1 V
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20 157 using an electrochemical workstation (VMP3, Bio-Logic Science Instruments, France)
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23 158 with Ag/AgCl as reference electrode. Electrochemical impedance spectroscopy (EIS)
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25 159 measurement was conducted over a frequency range of 100 kHz to 1 mHz with an AC
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28 160 signal of 10 mV amplitude. All electrolyte solutions were deaerated by high-purity
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30 161 nitrogen for at least 15 minutes prior to the measurement [32]. Coulombic efficiency
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33 162 (CE) was calculated as reported previously [4].

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36 163 Molecular biology analysis was performed to obtain the characteristics of the
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38 164 microbial population. Total genomic DNA was collected, pooled, and amplified
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40 165 according to previous procedures [35]. Then a mixture of amplicons was used for
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43 166 high-throughput 16S rRNA gene sequencing on MiSeq (Illumina, the USA). Raw
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46 167 sequencing data were deposited in the NCBI Sequence Read Archive with access
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48 168 number of SPR067096 and were analyzed according to Hao et al. [35].
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169 **3. Results and discussion**

170 **3.1 Characteristics of the modified anodes**

171 It was observed that the modified carbon-fiber-felt surfaces were covered with
172 dense layers, comprising vertically oriented nanosheets on top, which formed vertical
173 pores through the layer, compared with raw carbon-fiber-felt anode (Fig. 1a, Fig. 1b,
174 Fig. 1c). This structure improved the roughness of the anode surface, which was
175 similar with previous studies [17,18] and meant that the 3D open porous structure was
176 favorable to biofilm growth as well as permeability of electrolyte, substrates, and
177 electron mediators [36]. These vertically oriented nanosheets extending upward from
178 the surface of carbon-fiber-felts could provide direct pathways for electrons to transfer
179 from exoelectrogens in the biofilm to the carbon-fiber-felts. Moreover,
180 carbon-fiber-felts with larger specific surface areas were selected as the basic material
181 in present study instead of plain carbon paper, which would provide more sites for
182 microbes' attachment [17,18]. The compositions of the modified anodes were
183 examined using EDX and the corresponding elements (Ti, Fe, O) were detected (Fig.
184 1d), indicating that the anodes were well modified with metal oxides. It was
185 interesting to note that some metal oxides could not only enhance the interfacial
186 electron transfers in MFCs [17] but also stimulate the growth of chemoautotrophic
187 and heterotrophic bacteria using solar energy [37]. This implied that the proposed
188 modified anodes were promising and ready for following experiments.

3.2 Bioelectricity generation by the MFCs

Polarization curves were obtained using closed-circuit MFCs during 48 h operation (Fig. 2a). MFC-TiO₂ exhibited the highest maximum power density of 607.8 ± 16.1 mW m⁻² at current density of 1591.38 mA m⁻² with 150 Ω external resistances, followed by MFC-Fe₂O₃ with this value of 537.6 ± 14.8 mW m⁻² at current density of 1296.15 mA m⁻² with 200 Ω external resistances, 1.53 and 1.36 folds higher than the maximum power output of MFC-CF (396.1 ± 11.7 mW m⁻² with 400 Ω external resistances), while the maximum power output of MFC-CF was slightly higher than that obtained from previous study, where maximum power output of 283 mW m⁻² was obtained in dual chamber MFCs (300 mL net volume for anode chamber) with 300 mg L⁻¹ initial sulfide concentration [25]. The results indicated that the modified anodes with the TiO₂ and Fe₂O₃ nanosheets vertically oriented on the surface of carbon-fiber-felt could increase the power output of MFCs by providing more sites for microbes' attachment and more direct electrons transfer pathways [17,18].

Anode potentials which dominated the above difference were also monitored during this period (Fig. 2b). The anode potentials of the MFC-CF were lower than those obtained with sulfide free substrate due to lower redox potential of sulfide [38,39]. Additionally, the sulfide added to the anodic solution and the sulfate produced through sulfide oxidation acted as a soluble redox mediator, which could promote electron transfer from the bacterial cells to the anode surface [40]. The anode potentials of both MFC-TiO₂ and MFC-Fe₂O₃ were further lower than those of MFC-CF. It should be noted that previous results had shown that the lower negative

211 anode potential could demonstrate better activity of anode communities [41].
212 Moreover, metal oxides possess superior electronic, optical, and dielectric properties
213 and had been employed for surface modifications of anodes in MFCs for high power
214 output [36]. Hence, the present results indicated that carbon-fiber-felt with TiO_2 and
215 Fe_2O_3 nanosheets vertically oriented on the surface as anode materials could facilitate
216 adhesion, growth and activity of bacteria for enhancement of MFC performance.

217 The electrochemical behaviors of the modified anodes were also characterized.
218 More obvious redox peaks and much higher redox peak currents than untreated
219 carbon-fiber-felt anode were observed in CV curves, especially with TiO_2 nanosheets
220 (Fig. 3a). This indicated that electrodes with metal oxides nanosheets vertically
221 oriented on the surface of carbon-fiber-felt had better electron transfer properties than
222 an untreated electrode [42]. EIS was used to characterize the electrode surface and to
223 evaluate the kinetics of the electrochemical reaction and dramatically decrease of
224 internal resistances for MFCs with modified anodes were observed in Fig. 3b. Charge
225 transfer resistances derived from the MFC- TiO_2 and MFC- Fe_2O_3 were also
226 remarkably smaller than that of the untreated electrode, consistent with the CV
227 behavior and showing the advantage of the proposed modification in electron
228 transfers.

229 **3.3 Sulfide and organics removals in the MFCs**

230 Concentrations of sulfide and generated sulfate as well as TOC were monitored
231 initially and after 48 h operation (Fig. 4). Both sulfide and TOC declined after the
232 operation, demonstrating the feasibility of MFC technology for the removal of sulfide

and organic carbon simultaneously. Similar as the power outputs, sulfide removal efficiencies of MFC-TiO₂ ($94.4 \pm 1.5\%$) and MFC-Fe₂O₃ ($91.6 \pm 1.7\%$) were higher than that of MFC-CF ($88.5 \pm 1.9\%$), with the removal efficiency obtained from MFC-CF comparable with results from dual chamber MFCs that 84.7% of 100 mg L⁻¹ sulfide in the influent was removed within 72 h [4] and higher than removal efficiency of 60% with 80 mg L⁻¹ initial sulfide concentration in 72 h operation [26].

Nevertheless, sulfide removal efficiencies obtained in this study were relatively lower than those achieved by Cai et al. [43], where above 99% of added sulfide were removed, as our used cathode electron acceptor (K₃[Fe(CN)₆]) possesses lower redox potential than their employed KMnO₄ [24]. The TOC removal efficiencies also exhibited the similar principles, with MFC-TiO₂ ($56.9 \pm 1.8\%$) and MFC-Fe₂O₃ ($55.2 \pm 1.4\%$) higher than that of MFC-CF ($31.7 \pm 1.7\%$). The removal efficiencies of sulfide were superior to the TOC indicated that sulfide and organics acted as co-electron donors with competitive relationship and sulfide was easier to be oxidized than organic matter because of its lower redox potential [44,45]. Moreover, the calculated CE based on COD of the MFC-TiO₂ ($13.2 \pm 1.7\%$), MFC-Fe₂O₃ ($12.2 \pm 1.6\%$) and MFC-CF ($11.4 \pm 1.8\%$) was comparable with similar systems as previously reported [4]. This implied that carbon-fiber-felt with these two kinds of metal oxide (TiO₂ and Fe₂O₃) nanosheets vertically oriented on its surface as promising anode material could enhance both sulfide and organics removals in MFCs.

Oxidation products of sulfide in the MFCs were investigated. After 48 h operation, concentrations of generated sulfate in the exhausted anolyte were $3.26 \pm$

0.97 mg L⁻¹ in MFC-TiO₂ and 2.74 ± 0.89 mg L⁻¹ in MFC-Fe₂O₃, respectively (Fig. 4), with other soluble species (S₂O₃²⁻, SO₃²⁻) undetected in all MFCs, suggesting that most oxidation products were insoluble. In fact, many obvious solid particles covered on the anode surfaces after operation (Figure S1, Supporting Information). The particles were examined using EDX and it was found that the main component was elemental sulfur (Fig. 1d), which was the expected oxidation product as it was a non-corrosive solid and was easy to remove from aqueous solutions [4]. The present results suggested that elemental sulfur was the main product for sulfide removal when the electrochemical and biological oxidations were performed in MFCs, also proved by previous studies [4,46]. Recovery and quantification of generated elemental sulfurs were difficult as they mixed with biofilms. The relatively lower sulfate concentration and higher peak of elemental sulfur indicated that the modified carbon-fiber-felt electrodes were more amenable to biofilm growth, enabling more sulfur to be generated.

3.4 Identification of the involved microbes

The monitored voltage outputs and sulfide removals were relatively stable during the whole experiment. After 3 months operation, it was found that the biomass densities of the MFC-TiO₂ (26.7 µg cm⁻¹) and MFC-Fe₂O₃ (22.1 µg cm⁻¹) were higher than that of MFC-CF (16.2 µg cm⁻¹), suggesting more microbes adhering on the modified anodes (Figure S1, Supporting Information). Total numbers of operational taxonomy units (OTUs) estimated by Chao and Ace estimators with infinite sampling in MFC-TiO₂ and MFC-Fe₂O₃ were much larger than that in MFC-CF (Table 1),

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4 277 indicating that MFC-TiO₂ and MFC-Fe₂O₃ possessed greater richness of microbial
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6 278 diversity than MFC-CF. Both Simpson and Shannon diversity index provide not only
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8 279 the simply species richness (i.e., the number of species present) but how the
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10 280 abundance of each species is distributed (the evenness of the species) among all the
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12 281 species in the community. The increase of Shannon index and decrease of Simpson
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14 282 index of MFCs with modified anodes compared with raw one implied the bacterial
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16 283 communities in MFC-TiO₂ and MFC-Fe₂O₃ were more diverse than those in the
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18 284 MFC-CF due to the stimulated growth bacteria with the added metal oxides [36].
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23 285 Besides, visible light-excited photoelectrons from metal oxide could stimulate the
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25 286 growth of chemoautotrophic and heterotrophic bacteria [37].
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28 287 16S rRNA gene sequence and taxonomy analyses for the microbes in the three
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30 288 MFCs were performed at phylum, class and genus levels in order to understand the
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32 289 role of bacteria in enhanced sulfide oxidation and bioelectricity generation (Table 2).
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34 290 Electrochemically activated bacteria that were conducive to bioelectricity generation
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36 291 were enriched in the MFCs, especially with modified anodes. Bacteroidetes, the most
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38 292 frequently appeared species in the anode biofilms of MFCs with electrochemical
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40 293 activity as reported by Ha et al. [46] were enriched in MFC-TiO₂ (4.94%) and
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42 294 MFC-Fe₂O₃ (1.45%) than those in MFC-CF (1.22%). Moreover, with the
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44 295 enhancement of MFCs' functions of electricity generation and sulfide removal, plenty
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46 296 of bacteria with electrochemical activity were domesticated, such as the
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48 297 Deltaproteobacteria species, especially in the MFCs with modified anode. This
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50 298 implied that the design with TiO₂ and Fe₂O₃ nanosheets vertically oriented on the
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surface of carbon-fiber-felt could accumulate more electrochemically activated bacteria to generate bioelectricity than the untreated anode.

Sulfur related bacteria responsible for sulfide oxidation and sulfate reduction were also detected. *Thiobacillus* in Alphaproteobacteria, a famous sulfur-oxidizing bacterium that can oxidize sulfur to sulfate was enriched in MFC-TiO₂ [43,47]. *Spirochaeta* of Spirochaetes requiring sulfide in the growth medium and oxidizing it non-enzymically to elemental sulfur was found in MFC-Fe₂O₃ [48]. Sulfate-reducing bacteria were also greatly enhanced in the MFCs with the proposed anodes, such as *Desulfovibrio* genus of Deltaproteobacteria, which could reduce sulfate as well as other sulfur species such as sulfite and thiosulfate [4,23]. These species worked together to realized higher sulfide removals in the improved MFCs.

Pseudoxanthomonas of Gammaproteobacteria with an abundance of 4.63% in MFC-CF could be responsible for sulfide removals in this reactor as this species can oxidize sulfide to sulfate [49]. More species of sulfur related bacteria were found in the three MFCs than previously reported by Sun et al. [50] who employed sulfide as the sole electron donor in the MFCs; the greater variety and numbers of bacteria probably occurred due to the complex substrate (glucose and sulfide) employed in the present study.

Less anaerobic fermentative bacteria without electrochemical activity appeared in the MFCs with modified anodes. Firmicutes accounted for the largest portion of bacteria found in anaerobic sludge [51] decreased more greatly in MFC-TiO₂ and MFC-Fe₂O₃ than in MFC-CF. *Rhodobacter* sp. (belonging to Alphaproteobacteria)

promoting anaerobic fermentation [52] also exhibited the similar principles. These indicated that anaerobic fermentation process competing with bioelectricity generation was further weakened when MFCs equipped with carbon-fiber-felt anodes with TiO₂ and Fe₂O₃ nanosheets vertically oriented on their surfaces.

4. Conclusions

The surfaces of carbon-fiber-felt were successfully modified by layers of vertically oriented TiO₂ and Fe₂O₃ nanosheets respectively and acted as anodes in MFCs. Higher maximum power outputs of MFC-TiO₂ (607.75 mW m⁻²) and MFC-Fe₂O₃ (537.63 mW m⁻²) were obtained, compared with the MFC with untreated carbon-fiber-felt (396.05 mW m⁻²), and the sulfide and TOC removal efficiencies also increased. These results could be contributed to more active sites for microbial adhesion with increasing biomass densities by the modified anodes. Increases of microbial diversities were also observed by high-throughput 16S rRNA gene sequencing analysis and specific functional species were found, such as the enhanced Bacteroidetes responsible for bioelectricity generation with *Thiobacillus* and *Spirochaeta* dominating sulfide removal, with less anaerobic fermentative bacteria Firmicutes.

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References

1. Celis-García LB, González-Blanco G, Meraz M. Removal of sulfur inorganic compounds by a biofilm of sulfate reducing and sulfide oxidizing bacteria in a down-flow fluidized bed reactor. *J Chem Technol Biotechnol*. 2008;83:260–268.
2. Long JW, Wallace JM, Peterson GW, Huynh K. Manganese oxide nanoarchitectures as broad-spectrum sorbents for toxic gases. *ACS Appl Mater Interfaces*. 2016;8:1184–1193.
3. Kim KH. The need for practical input data for modeling odor nuisance effects due to a municipal solid waste landfill in the surrounding environment. *Environ Internat*. 2016;87:116–117.
4. Zhang BG, Zhao HZ, Shi CH, Zhou SG, Ni JR. Simultaneous removal of sulfide and organics with vanadium (V) reduction in microbial fuel cells. *J Chem Technol Biotechnol*. 2009;84:1780–1786.
5. Henshaw PF, Zhu W. Biological conversion of hydrogen sulphide to elemental sulphur in a fixed-film continuous flow photo-reactor. *Water Res*. 2001;35:3605–3610.
6. Cirne DG, Van Der Zee FP, Fernandez-Polanco M, Fernandez-Polanco F. Control of sulphide during anaerobic treatment of S-containing wastewaters by adding limited amounts of oxygen or nitrate. *Rev Environ Sci Biotechnol*. 2008;7:93–105.
7. Lin HJ, Williams N, King A, Hu B. Electrochemical sulfide removal by low-cost electrode materials in anaerobic digestion. *Chem Eng J*. 2016; 297:180–192.

- 1
2
3
4 363 8. Penteado ED, Fernandezmarchante CM, Zaiat M, Gonzalez ER, Rodrigo MA.
5
6 364 Influence of carbon electrode material on energy recovery from winery
7
8 365 wastewater using a dual chamber microbial fuel cell. *Environ Technol.*
9
10 366 2017;38:1333–1341.
11
12
13 367 9. Du Q, An JK, Li JH, Zhou L, Li N, Wang X. Polydopamine as a new modification
14
15 368 material to accelerate startup and promote anode performance in microbial fuel
16
17 369 cells. *J Power Sources*. 2017;343:477–482.
18
19
20 370 10. Hao LT, Zhang BG, Tian CX, Liu Y, Shi CH, Cheng M, Feng CP. Enhanced
21
22 371 microbial reduction of vanadium (V) in groundwater with bioelectricity from
23
24 372 microbial fuel cells. *J Power Sources*. 2015;287:43–49.
25
26
27 373 11. Liu JD, Liu LF, Gao B. The tubular MFC with carbon tube air-cathode for power
28
29 374 generation and N,N-dimethylacetamide treatment. *Environ Technol.*
30
31 375 2016;37:762–767.
32
33
34 376 12. Yamashita T, Ishida M, Ogino A, Yokoyama H. Evaluation of organic matter
35
36 377 removal and electricity generation by using integrated microbial fuel cells for
37
38 378 wastewater treatment. *Environ Technol*. 2016;37(2):228–236.
39
40
41 42 379 13. Lorenzo MD, Scott K, Curtis TP, Head IM. Effect of increasing anode surface
43
44 380 area on the performance of a single chamber microbial fuel cell. *Chem Eng J*.
45
46 381 2010;156:40–48.
47
48
49 382 14. Kumar GG, Sarathi VG, Nahm KS. Recent advances and challenges in the anode
50
51 383 architecture and their modifications for the applications of microbial fuel cells.
52
53 384 *Biosens Bioelectron*. 2013;43:461–475.
54
55
56
57
58
59
60

1
2
3
4 385 15. Sivasankaran A, Sangeetha D, Ahn YH. Nanocomposite membranes based on
5
6 386 sulfonated polystyrene ethylene butylene polystyrene (SSEBS) and sulfonated
7
8 387 SiO₂ for microbial fuel cell application. Chem Eng J.2016;289:442–451.
9
10
11 388 16. Tang JH, Yuan Y, Liu T, Zhou SG. High-capacity carbon-coated titanium dioxide
12
13 389 core-shell nanoparticles modified three dimensional anodes for improved energy
14
15 390 output in microbial fuel cells. J Power Sources. 2015;247:170–176.
16
17
18 391 17. Yin T, Lin ZY, Su L, Yuan CW, Fu DG. Preparation of vertically oriented TiO₂
19
20 392 nanosheets modified carbon paper electrode and its enhancement to the
21
22 393 performance of MFCs. ACS Appl Mater Interfaces. 2015;7:400–408.
23
24
25 394 18. Wang L, Su L, Chen HH, Yin T, Lin ZY, Lin XX, Yuan CW, Fu DG. Carbon paper
26
27 395 electrode modified by goethite nanowhiskers promotes bacterial extracellular
28
29 396 electron transfer. Mater Lett. 2015;141:311–314.
30
31
32 397 19. Hidalgo D, Tommasi T, Bocchini S, Chiolerio A, Chiodoni A, Mazzarino I,
33
34 398 Ruggeri B. Surface modification of commercial carbon felt used as anode for
35
36 399 microbial fuel cells. Energy. 2016;99:193–201.
37
38
39 400 20. Liu H, Ramnarayanan R, Logan BE. Production of electricity during wastewater
40
41 401 treatment using a single chamber microbial fuel cell. Environ Sci Technol.
42
43 402 2004;38:2281–2285.
44
45
46 403 21. Zhu NW, Chen X, Zhang T, Wu PX, Li P, Wu JH. Improved performance of
47
48 404 membrane free single-chamber air-cathode microbial fuel cells with nitric acid
49
50 405 and ethylenediamine surface modified activated carbon fiber felt anodes.
51
52
53 406 Bioresour Technol. 2011;102:422–426.
54
55
56
57
58
59
60

- 1
2
3
4 407 22. Cai J, Zheng P. Simultaneous anaerobic sulfide and nitrate removal in microbial
5
6 408 fuel cell. *Bioresour Technol.* 2013;128:760–764.
7
8
9 409 23. Zhao F, Rahunen N, Varcoe JR, Avignone-Rossa C, Thumser AE, Slade RCT.
10
11 410 Factors affecting the performance of microbial fuel cells for sulfur pollutants
12
13 411 removal. *Biosens Bioelectron.* 2009;24:1931–1936.
14
15
16 412 24. Fatemi S, Ghoreyshi AA, Rahimnejad M, Darzi GN, Pant D. Sulfide as an
17
18 413 alternative electron donor to glucose for power generation in mediator-less
19
20 414 microbial fuel cell. *J Environ Sci Health Part A.* 2017;52:1150–1157.
21
22
23 415 25. Cai J, Zheng P, Qaisar M, Xing YJ. Effect of operating modes on simultaneous
24
25 416 anaerobic sulfide and nitrate removal in microbial fuel cell. *J Ind Microbiol*
26
27 417 *Biotechnol.* 2014;41:795–802.
28
29
30 418 26. Rakoczy J, Feisthauer S, Wasmund K, Bombach P, Neu TR, Vogt C, Richnow HH.
31
32 419 Benzene and sulfide removal from groundwater treated in a microbial fuel cell.
33
34 420 *Biotechnol Bioeng.* 2013;110:3104–3113.
35
36
37 421 27. Zhang L, Mao Y, Ma J, Li D, Shi H, Liu Y, Cai L. Effect of the chemical
38
39 422 oxidation demand to sulfide ratio on sulfide oxidation in microbial fuel cells
40
41 423 treating sulfide-rich wastewater. *Environ Technol.* 2013;34:269–274.
42
43
44 424 28. Cai J, Zheng P, Qaisar M, Sun P. Effect of electrode types on simultaneous
45
46 425 anaerobic sulfide and nitrate removal in microbial fuel cell. *Sep Purif Technol.*
47
48 426 2014;134:20–25.
49
50
51 427 29. Zhang BG, Zhou SG, Zhao HZ, Shi CH, Kong LC, Sun JJ, Ni JR. Factors
52
53 428 affecting the performance of microbial fuel cells for sulfide and vanadium (V)
54
55
56
57
58
59
60

429 treatment. *Bioproc Biosyst Eng.* 2010;33:187–194.

430 30. Liu HP, Zhang BG, Liu Y, Wang ZJ, Hao LT. Continuous bioelectricity generation
431 with simultaneous sulfide and organics removals in an anaerobic baffled stacking
432 microbial fuel cell. *Int J Hydrogen Energy.* 2015;40:8128–8136.

433 31. Cline JD. Spectrophotometric determination of hydrogen sulphide in natural
434 waters. *Limnol Oceanogr.* 1969;14:454–458.

435 32. Wei JC, Liang P, Cao XX, Huang X. A new insight into potential regulation on
436 growth and power generation of *Geobacter sulfurreducens* in microbial fuel cells
437 based on energy viewpoint. *Environ Sci Technol.* 2010;44:3187–3191.

438 33. Xiao B, Han Y, Liu X, Liu J. Relationship of methane and electricity production
439 in two-chamber microbial fuel cell using sewage sludge as substrate. *Int J*
440 *Hydrogen Energy.* 2014;39:16419–16425.

441 34. Logan BE, Hamelers B, Rozendal R, Schröder U, Keller J, Freguia S, Aelterman
442 P, Verstraete W, Rabaey K. Microbial fuel cells: methodology and technology.
443 *Environ Sci Technol.* 2006;40:5181–5192.

444 35. Hao LT, Zhang BG, Cheng M, Feng CP. Effects of various organic carbon sources
445 on simultaneous V(V) reduction and bioelectricity generation in single chamber
446 microbial fuel cells. *Bioresour Technol.* 2016;201:105–110.

447 36. Qiao Y, Bao SJ, Li CM, Cui X Q, Lu ZS, Guo J. Nanostructured
448 polyaniline/titanium dioxide composite anode for microbial fuel cells. *ACS Nano.*
449 2008;2:113–119.

450 37. Lu AH, Li Y, Jin S, Wang X, Wu XL, Zeng CP, Li Y, Ding HR, Hao RX, Lv M,

- 451 Wang CQ, Tang YQ, Dong HL. Growth of non-phototrophic microorganisms
452 using solar energy through mineral photocatalysis. Nat Commun. 2012;3:768.
- 453 38. Min B, Cheng S, Logan BE. Electricity generation using membrane and salt
454 bridge microbial fuel cells. Water Res. 2005;39:1675–1686.
- 455 39. Bond D R, Lovley D R. Electricity production by *Geobacter*
456 *sulfurreducens* attached to electrodes. Appl Environ Microbiol.
457 2003;69:1548–1555.
- 458 40. Ieropoulos I, Greenman J, Melhuish C, Hart J. Energy accumulation and improved
459 performance in microbial fuel cells. J Power Sources. 2005;145:253–256.
- 460 41. Aelterman P, Freguia S, Keller J, Verstraete W, Rabaey K. The anode potential
461 regulates bacterial activity in microbial fuel cells. Appl Microbiol Biotechnol.
462 2008;78:409–418.
- 463 42. Zhou MH, Chi M, Wang H, Jin T. Anode modification by electrochemical
464 oxidation: a new practical method to improve the performance of microbial fuel
465 cells. Biochem Eng J. 2012;60:151–155.
- 466 43. Cai J, Zheng P, Xing Y, Qaisar M. Effect of electricity on microbial community of
467 microbial fuel cell simultaneously treating sulfide and nitrate. J Power Sources.
468 2015;281:27–33.
- 469 44. Raschitor A, Soreanu G, Fernandez-Marchantea CM, Lobatoa J, Cañizaresa P,
470 Cretescu I, Rodrigo MA. Bioelectro-Claus processes using MFC technology:
471 Influence of co-substrate. Bioresour Technol. 2015;189:94–98.
- 472 45. Zhang J, Zhang BG, Tian CX, Ye ZF, Liu Y, Lei Z, Feng CP. Simultaneous sulfide

- removal and electricity generation with corn stover biomass as co-substrate in microbial fuel cells. *Bioresour Technol.* 2013;138:198–203.
46. Ha PT, Lee TK, Rittmann BE, Park J, Chang IS. Treatment of Alcohol Distillery Wastewater Using a Bacteroidetes-Dominant Thermophilic Microbial Fuel Cell. *Environ Sci Technol.* 2012;46:3022–3030.
47. Kellermann C, Griebler C. *Thiobacillus thiophilus* sp. nov., a chemolithoautotrophic, thiosulfate-oxidizing bacterium isolated from contaminated aquifer sediments. *Int J Syst Evol Micro.* 2009;59:583–588.
48. Dubinina G, Grabovich M, Leshcheva N, Rainey FA, Gavrish E. *Spirochaeta perfilievii* sp. nov. an oxygen-tolerant, sulfide-oxidizing, sulfur- and thiosulfate-reducing spirochaete isolated from a saline spring. *Int J Syst Evol Microbiol.* 2011;61:110–117.
49. Krishnani KK, Kathiravan V, Natarajan M, Kailasam M, Pillai SM. Diversity of Sulfur-oxidizing bacteria in greenwater system of coastal aquaculture. *Appl Biochem Biotechnol.* 2010;162:1225–1237.
50. Sun M, Tong ZH, Sheng GP, Chen YZ, Zhang F, Mu ZX, Wang HL, Zeng RJ, Liu XW, Yu HQ, Wei L, Ma F. Microbial communities involved in electricity generation from sulfide oxidation in a microbial fuel cell. *Biosens Bioelectron.* 2010;26:470–476.
51. Park HS, Kim BH, Kim HS, Kim HJ, Kim GT, Kim M, Chang IS, Park YK, Chang HI. A novel electrochemically active and Fe(III)-reducing bacterium phylogenetically related to *Clostridium butyricum* isolated from a microbial fuel

cell. *Anaerobe*. 2001;7:297–306.

52. Srinivas TNR, Kumar PA, Sasikala C, Ramana CV, Imhoff JF. *Rhodobacter vinaykumarii* sp. nov., a marine phototrophic alphaproteobacterium from tidal waters, and emended description of the genus *Rhodobacter*. *Int J Syst Evol Micr*. 2007;57:1984–1987.

Figure Captions.

Fig. 1. SEM images and EDX spectral intensities. (a) with TiO₂ nanosheets, (b) with Fe₂O₃ nanosheets, (c) bare carbon-fiber-felt, (d) corresponding EDX spectral intensities.

Fig. 2. (a) Polarization curves and power outputs as well as (b) anode potentials of MFCs with three kinds of anodes.

Fig. 3. (a) CV and (b) Nyquist plot of EIS data for the anodes of three MFCs.

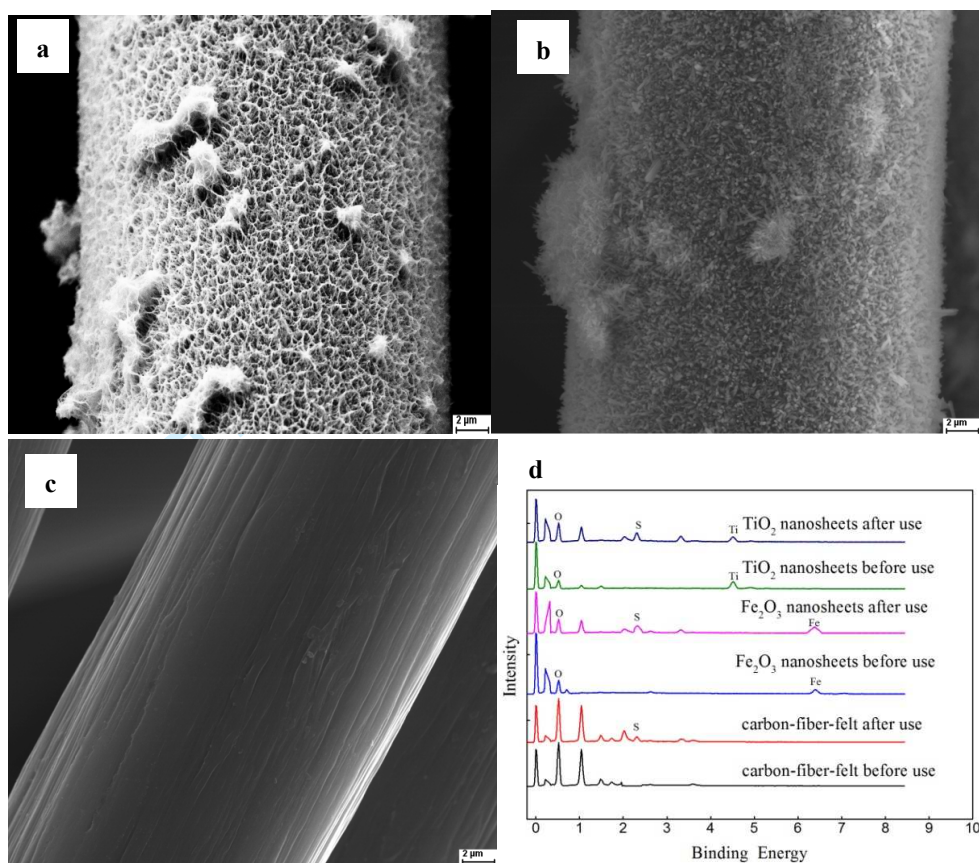
Fig. 4. Changes of sulfide and TOC during 48 h operation as well as the generated sulfate in the anode chambers of three MFCs. The black legend referred to left vertical axis while the red legend referred to the right vertical axis.

512 **Table 1.** Alpha-diversity of three MFCs employed in this study.

Type	Ace	Chao	Shannon	Simpson	Coverage
MFC-TiO ₂	768	760	4.33	0.031	0.996
MFC-Fe ₂ O ₃	505	493	2.69	0.192	0.996
MFC-CF	394	375	2.45	0.240	0.995

Table 2. Percentages of sequences identified to different phylogenies in the MFCs with three different anodes.

Phylum	Class	Genus	MFC-TiO ₂ (%)	MFC-Fe ₂ O ₃ (%)	MFC-CF (%)	Phylum	Class	Genus	MF C-Ti O ₂ (%)	MFC-Fe ₂ O ₃ (%)	MFC-CF (%)
Acidobacteria	Acidobacteria	norank	0.12	0.17	0.32	Nitrospirae	Nitrospira	norank	0.21	0.01	0.28
	Holophagae	norank	5.30	5.55	0.00			<i>Gemmata</i>	0.73	0.68	0.00
	Actinobacteria	uncultured	0.40	0.15	0.46	Planctomycetes	Planctomycetacia	<i>Pirellula</i>	0.96	0.42	1.31
	Thermoleophilia	<i>Gaiella</i>	0.30	0.17	0.44			<i>Planctomyces</i>	1.81	1.75	0.00
Armatimonadetes	norank	norank	0.07	0.08	0.07			uncultured	0.47	0.20	1.03
	Bacteroidia		4.41	1.03	0.71			norank	0.18	0.14	0.30
Bacteroidetes	Sphingobacteriia	norank	0.42	0.03	0.00		Alphaproteobacteria	<i>Rhodobacter</i>	0.04	0.02	14.8
	Sphingobacteriia		0.00	0.22	0.48			<i>Delftia</i>	0.78	1.47	1.41
	vadinHA17	norank	0.11	0.17	0.03			<i>Thiobacillus</i>	1.21	0.01	0.00
Candidate division						Proteobacteria					
BRC1	norank	norank	1.94	0.46	0.25		Deltaproteobacteria	<i>Desulfovibrio</i>	12.97	6.86	4.30
	Anaerolineae	<i>Leptolinea</i>	0.88	0.12	0.00			norank	21.94	47.44	28.6
Chloroflexi							Gammaproteobacteria	<i>Pseudoxanthomonas</i>	0.90	0.00	4.63
	Caldilineae	uncultured	0.98	0.43	0.42						
		<i>Anaerofustis</i>	0.01	0.00	0.17	Spirochaetae	Spirochaetes	<i>Spirochaeta</i>	0.10	0.05	0.11
Firmicutes	Clostridia	<i>Incertae_Sedis</i>	0.94	0.05	37.88	Synergistetes	Synergistia	uncultured	3.75	0.09	0.02
	Negativicutes	norank	37.26	31.73	0.03	Others			0.81	0.50	1.95

**Figure 1**

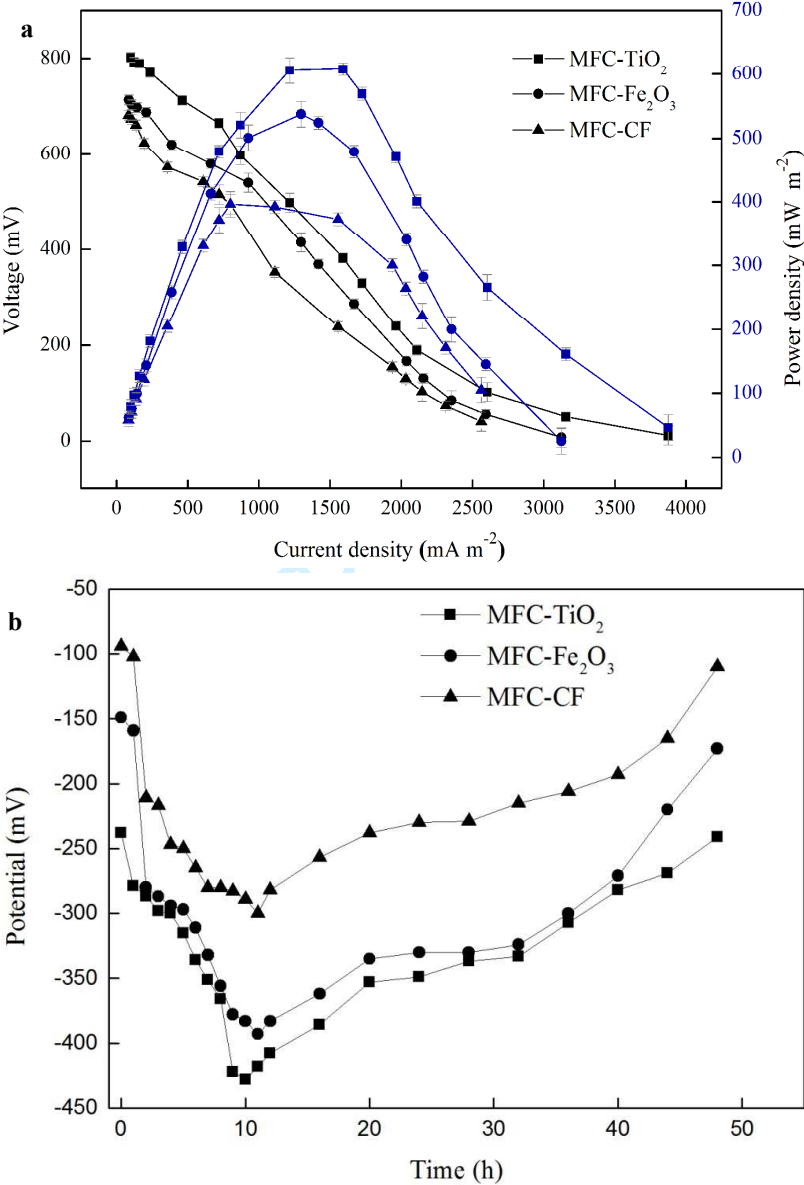
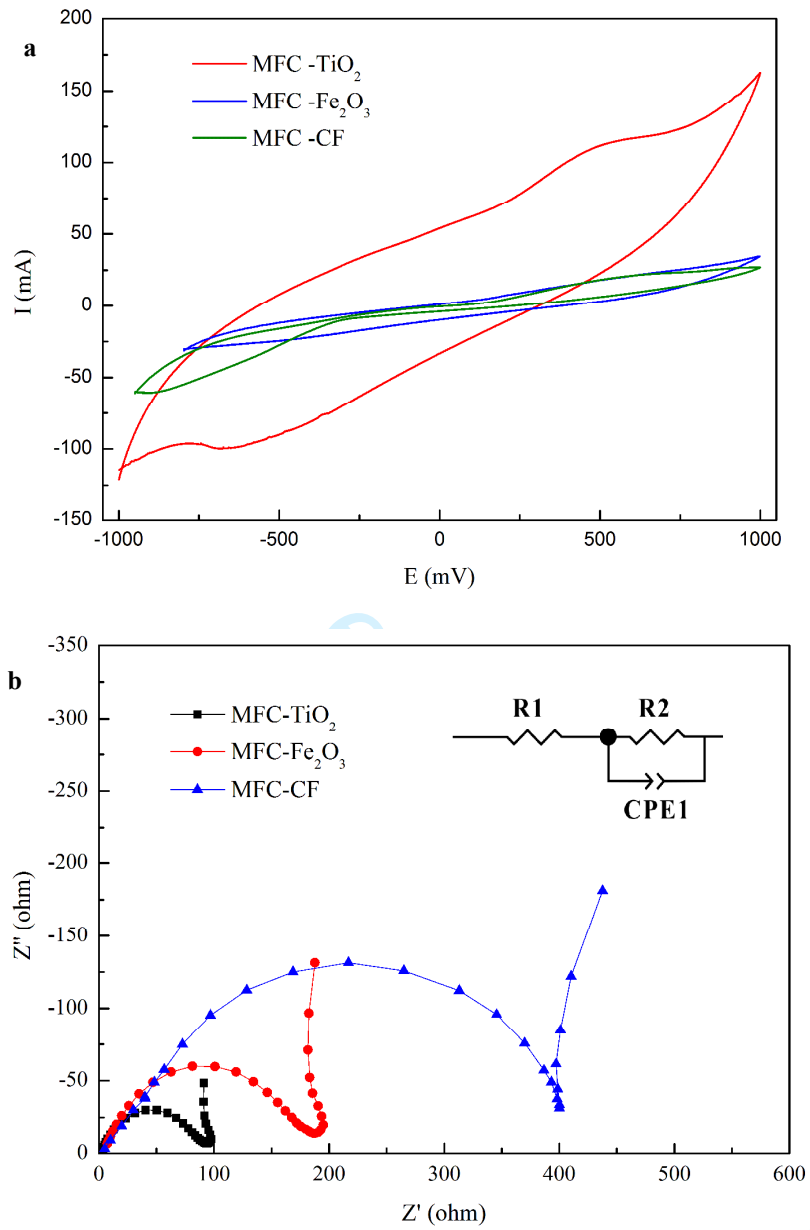


Figure 2

**Figure 3**

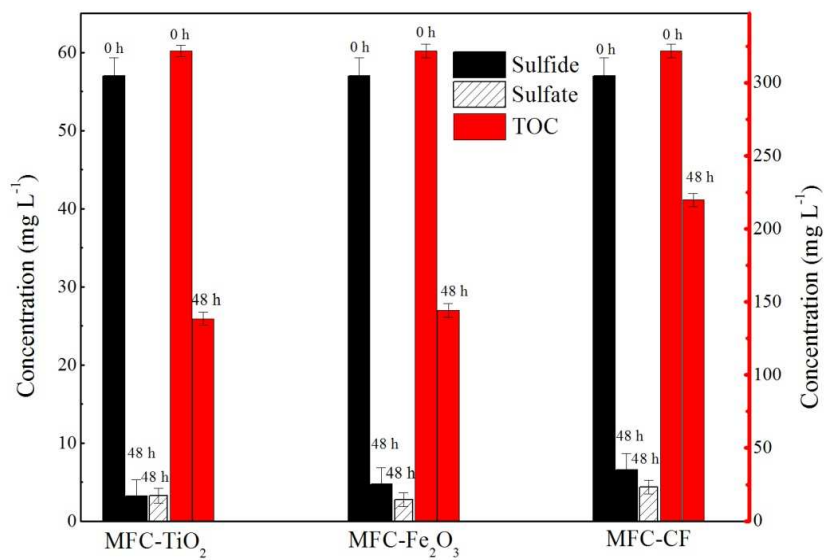


Figure 4

Supporting Information

Enhanced sulfide removal and bioelectricity generation in microbial fuel cells with anodes modified by vertically oriented nanosheets

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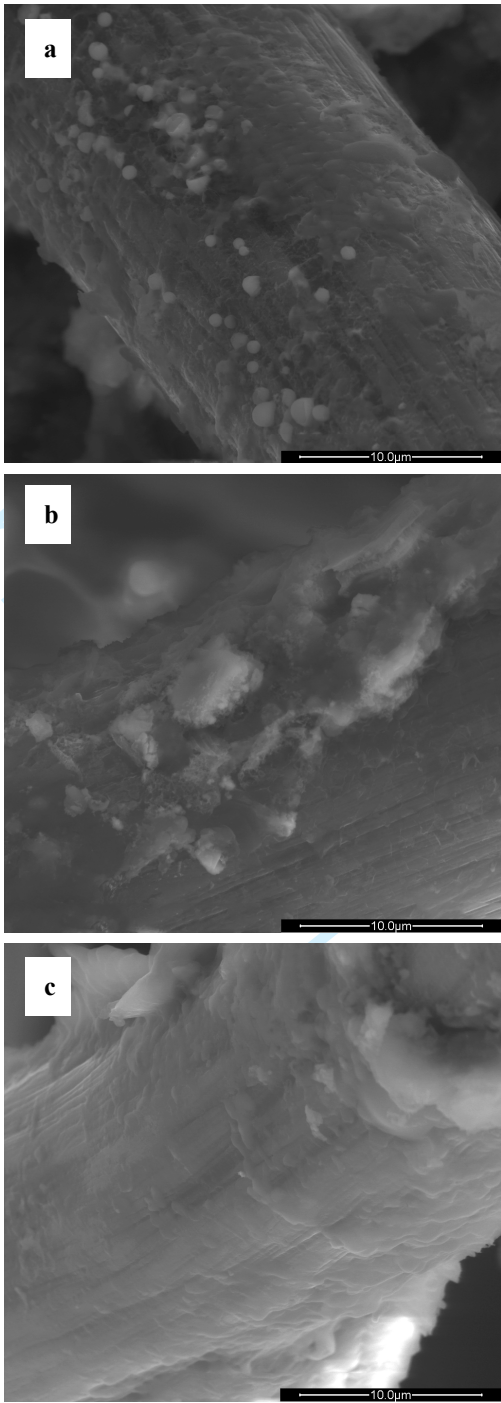


Figure S1. SEM images of anodes in the three MFCs after the whole experiment. (a) MFC-TiO₂; (b) MFC-Fe₂O₃; (c) MFC-CF.